Strategies to improve outcome after partial liver resection

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Chapter 1

Introduction and outline of the thesis
In this thesis, two important complications of partial liver resections are addressed, namely 1) liver failure, caused by inadequate function of the remnant liver, and 2) hepatic ischemia and reperfusion injury, caused by vascular inflow occlusion, which may increase the risk of remnant liver failure. **Part 1** (chapter 2-6) consists of clinical studies on outcome of partial liver resections and prevention of liver insufficiency and liver failure. **Part 2** (chapter 7-9) consists of experimental studies aiming at increasing remnant liver volume or decreasing liver damage and inflammation secondary to hepatic ischemia and reperfusion. **Part 3** (chapter 10-12) consists of experimental studies on hypothermic perfusion to decrease hepatic ischemia and reperfusion injury.

**PART 1**

1.1 Liver resection

Partial liver resection is the only curative option for patients with liver malignancies, such as metastases from colorectal cancer, hepatocellular carcinoma or hilar cholangiocarcinoma. When performing partial liver resection, the anatomic division of the liver into segments according to Couinaud's classification is used (Fig. 1). The classical types of anatomical resection comprise segmentectomy (one segment), bisegmentectomy (2 segments), left hemihepatectomy (segments 1-4), right hemihepatectomy (segments 5-8) or extended right hemihepatectomy (segments 4-8). Recently, a new terminology of liver anatomy and resections has been proposed by the terminology committee of the International Hepato-Pancreato-Biliary Association

![Figure 1. The anatomical division of the liver into segments according to Couinaud](image-url)
morbidity is still substantial \(^2\)\(^4\). Most frequent postoperative complications are biliary leakage, infectious complications such as perihepatic abscess or wound infection, pleural effusion and bleeding \(^3\). Especially when liver function is compromised before operation due to cirrhosis, chronic sclerosing cholangitis or steatosis, the risk of major complications is increased \(^4\)\(^7\). An important cause of postoperative morbidity and mortality is loss of functional liver tissue due to resection. When more liver parenchyma is resected, the risk of postoperative liver insufficiency and liver failure increases \(^3\)\(^8\). Especially when postoperative liver failure is accompanied by infectious complications, intensive care treatment is often necessary and maximum median mortality rates are 70%. One group of patients that has an increased risk of postoperative complications after partial liver resection are patients with hilar cholangiocarcinoma \(^9\). These carcinomas originate from the proximal bile duct and often infiltrate into the liver. Forty to sixty percent of all cholangiocarcinomas arise from the proximal bile duct whereas other forms include intrahepatic cholangiocarcinomas (10-15%) and distal extrahepatic cholangiocarcinomas (20-30%) \(^10\). Hilar cholangiocarcinomas are also called Klatskin tumors, named after the person who first described a patient series in 1965 \(^11\). Klatskin Tumors can be classified as proposed by Bismuth and Corlette et. al. \(^12\). Only a minority of patients can be treated with curative intent, because at presentation the tumor often has metastasized or ingrown into the liver is too extensive \(^10\)\(^13\)\(^15\). For most type II and all type III tumors, treatment consists of hilar resection combined with partial liver resection. When ingrowth into the main trunk of the portal vein or hepatic artery is present, vascular resection is necessary in combination with hilar resection \(^16\)\(^17\). In selected cases, type IV tumors can also be resected depending on biliary segmental anatomy at the liver hilum. In the last 10 years, a more aggressive surgical strategy has been advocated, mainly by Japanese surgeons, in order to achieve a higher rate of tumor free margins and consequently, improved survival \(^14\)\(^17\)\(^21\). However, postoperative morbidity and mortality rates are high, ranging from 37%-85% and 0%-15% respectively \(^6\)\(^22\)\(^28\). In chapter 2, a series of 99 patients is described, which had undergone resection for Klatskin tumors in the Academic Medical Center in a 15 year period. Three periods of 5 years are compared in respect with postoperative morbidity and mortality and long-term survival. In chapter 3, an overview is given of the diagnostic and surgical approaches as applied in the Academic Medical Center. The criteria for resectability are discussed in the light of classification systems. Liver segment one, or the caudate lobe is often infiltrated in hilar cholangiocarcinoma patients due to its location directly posterior to the hepatic hilum. Concomitant complete resection of the caudate lobe is therefore important to obtain margin negative resections \(^19\)\(^27\)\(^29\). In chapter 4, the benefit of total resection of segment one to obtain tumor free resection margins during resection for Klatskin tumors is demonstrated in the same series of patients.

### 1.3 Assessment of liver functional reserve

As stated above, the volume of resected liver parenchyma is an important factor in the development of postoperative liver failure. When liver function is normal, and the patient is not diabetic or immunocompromised, up to 70% of liver volume can be resected without
increased risk of liver failure. On the other hand, when liver function is compromised, due to underlying liver disease, the safe resection limit lies around 50-60%. Total liver volume and volume of the liver segments that remain after resection (future remnant liver) can be calculated using computer tomography (CT)-volumetry. This technique is widely used to exclude patients from liver resection or to select patients who will benefit from a future remnant liver volume increasing procedure such as portal vein embolization. CT-volumetry is performed by manually delineating the whole liver, the tumor and the future remnant liver on preoperative CT abdominal images. A drawback of this technique is that it is based on liver morphology and provides no information on function of the liver. Therefore, measurement of remnant liver volume alone is not enough to assess resectability, especially in patients with parenchymal disease.

Liver function can be assessed with conventional laboratory tests such as bilirubin, albumin, and coagulation parameters. Many investigators, however, did not find any predictive value using these tests. Serum hyaluronic acid concentration is thought to reflect the degree of hepatic fibrosis and sinusoidal endothelial cell damage. Exogenous hyaluronic acid administered in an experimental setting, can be used to measure sinusoidal endothelial cell function. Hyaluronic acid has been found to correlate with conventional liver function tests and indocyanine green clearance rate. Indocyanine green is an organic dye that shows rapid liver uptake and is excreted into the bile by the adenosine 5'-triphosphate dependant export pump multidrug-resistance associated protein-2, without undergoing biotransformation. Indocyanine green clearance rate has shown predictive value for posthepatectomy liver failure. It is used in many centers, particularly in the Far East, to measure pre-operative liver function. A drawback of the above-mentioned liver function tests is that they provide no objective criteria concerning the amount of liver parenchyma that can safely be resected in any individual patient.

One method to measure functional distribution of the liver is technetium-99m labeled (99mTc)-galactosyl human serum albumin liver scintigraphy. It has been used as a receptor-targeted liver function test. A good correlation with indocyanine green clearance and conventional liver function parameters was found. In 2 studies, it also seems to have predictive value for short-term outcome. As a drawback, 99mTc-galactosyl human serum albumin accumulates within the liver and is not excreted into the bile. Therefore, no information is provided regarding biliary function. Furthermore, 99mTc-galactosyl human serum albumin is only available in Japan and not in Europe or the USA.

### 1.3.2 Hepatobiliary scintigraphy

Function of the future remnant liver can be measured using hepatobiliary scintigraphy with 99mTc-iminodiacetic acid analogues such as 99mTc-mebrofenin. Once injected into the blood stream, 99mTc-mebrofenin is totally cleared by the liver over time. The percentage of injected 99mTc-mebrofenin that is cleared within a certain period of time is therefore a measure of liver function. 99mTc-mebrofenin is excreted into the bile by the adenosine 5'-triphosphate-dependent export pumps, multidrug-resistance-associated protein 1 and 2, without undergoing biotransformation during transit through the hepatocyte. Preoperative 99mTc-mebrofenin clearance rates have shown to correlate with indocyanine green clearance rates in a series of partial hepatectomy patients. The advantage of hepatobiliary scintigraphy over other liver function tests is that 2-dimensional images of the liver are acquired with a gamma camera.
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showing the regional uptake and excretion of $^{99m}$Tc-mebrofenin by the liver. $^{99m}$Tc-mebrofenin uptake of the future remnant liver is calculated by manually drawing regions of interest around the future remnant liver and calculating radioactivity within this region. This is then divided by total liver $^{99m}$Tc-mebrofenin uptake and corrected for body surface area. In chapter 5, preoperative $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy is used to assess function of the future remnant liver in patients who would undergo partial liver resection. It is compared to hepatobiliary scintigraphy, measured 1-3 days after the operation to assess correlation of the results of both measurements. In chapter 6, a larger patient series is used to assess the predictive value of future remnant liver function measured with $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy. This method is compared to calculation of the future remnant liver by CT-volumetry and is related to postoperative morbidity, liver failure and mortality.
PART 2

2.1 Portal vein embolization

When the future remnant liver is too small, usually after planned extended right hemihepatectomy, partial liver resection cannot be performed because of the high risk of postoperative liver failure. For some of these patients, portal vein embolization can be considered. With this technique, usually the right main branch of the portal vein is completely embolized. This is most often performed using coils to close the large tributaries of the portal vein and polyvinyl alcohol to close the smaller vessels. Because portal venous flow to the embolized liver segments is blocked, these segments show atrophy and shrink. The other liver segments will receive more venous blood and therefore will show hypertrophy and increase in size. Thus, through a process of regeneration it is possible to augment size and function of the future remnant liver, making resection possible.

Tumor progression plays a crucial role in application of portal vein embolization, as previous reports show that the volume of metastatic liver tumors increases more rapidly than the volume of the liver parenchyma after portal vein embolization, both in nonemembolized and embolized liver segments. This acceleration is probably due to increased arterial flow in the embolized liver segments as most tumors mainly receive arterial blood supply. Therefore, dual embolization of hepatic artery and portal vein has been suggested to induce liver regeneration on the one hand and prevent stimulation of tumor growth before hepatectomy on the other hand. The obvious advantage of dual embolization compared to portal vein embolization only is, that complete occlusion of both portal and arterial blood supply to the tumor bearing liver segments is accomplished. The timing of portal vein embolization and arterial embolization is important, since simultaneous embolization increases the risk of parenchymal infarction. Also, no studies are available assessing the potential systemic and local proinflammatory cytokine response directly after dual embolization. In chapter 7, the effect of portal vein ligation as well as both portal vein and hepatic artery ligation, using two different time schedules, was studied in a rat model (Fig. 2). Outcome parameters were atrophy and hypertrophy as well as hepatocellular damage and inflammation.

2.2 Vascular inflow occlusion and postischemic reperfusion injury

Next to size and function of the FRL, another important determinant of the outcome of partial liver resections is blood loss during and after surgery. Several studies have shown that intra-operative blood loss is a significant prognostic factor for outcome after resection. To reduce blood loss, blood flow to the liver can be temporarily occluded during partial liver resection. This maneuver, first described by Pringle in 1885 (Pringle's maneuver), significantly reduces intra-operative blood loss. When backflow from the hepatic veins still causes major blood loss or when tumor is situated near the caval vein, total vascular exclusion can
be applied in which the supra- and infrahepatic caval vein are clamped in addition to the portal vein and hepatic artery. A major drawback of these clamping techniques during partial liver resection is that they induce liver ischemia, when applied for prolonged periods. In humans, signs of ischemia occur when clamping for 60 minutes or more. Ischemia can be defined as 'a condition in which an organ is deprived of sufficient blood supply'. It is accompanied by lack of oxygen and substrates, the essential sources for cellular energy metabolism, and failure to remove end-products of metabolism. This will lead to increased lactate production and therefore, metabolic acidosis due to anaerobic glycolysis. The lack of adenosine 5'-triphosphate as energy substrate, which is needed for maintenance of cell homeostasis and integrity, will cause cellular dysfunction, cellular and interstitial edema and eventually massive cell death (i.e. necrosis).

When removing the clamps after parenchymal dissection, the remnant liver is reperfused. Reperfusion has important benefits such as restoration of oxygen supply and removal of toxic metabolites. On the other hand, the combination of ischemia followed by reperfusion paradoxically enhances damage and inflammation. Two different phases of ischemia and reperfusion injury can be distinguished. An early phase (< 2 hours after reperfusion) in which production of reactive oxygen species plays a dominant role and a late phase (6 to 48 hours after reperfusion), characterized by the onset of an inflammatory response. These two phases result in enhanced parenchymal cell death with leakage of intracellular enzymes such as aspartate aminotransferase and alanine aminotransferase as well as increased apoptosis and necrosis.
2.2.2 Early phase of ischemia and reperfusion injury

Reactive oxygen species are molecules centered on oxygen that react rapidly and rather indiscriminately with all important classes of biological macromolecules. Under physiologic conditions, reactive oxygen species are generated in mitochondria of every cell. Under ischemic conditions, production is increased due to the enzyme xanthine oxidase. The latter is formed by conversion of xanthine dehydrogenase during ischemia. Xanthine oxidase catalyses a reaction forming hydrogen peroxide and superoxide anion, which are important reactive oxygen species. The reaction uses oxygen as a substrate, which is absent during ischemia, but present in abundance at reperfusion. Reactive oxygen species not only cause direct cellular damage, but also act as intracellular signaling molecules. They interact with target molecules including nuclear factor-kappa Beta and stress-activated protein kinases, implicated in apoptosis. Transcription factor Nuclear factor-kappa Beta plays a pivotal role in the onset of inflammatory reactions by inducing transcription of proinflammatory mediators.

The pathophysiological relevance of intracellular reactive oxygen formation during hepatic ischemia and reperfusion is a topic of debate. More evidence exists on extracellular reactive oxygen species formation as an important cause of damage. Known sources of extracellular reactive oxygen species producing cells are Kupffer cells, neutrophils and monocytes. Most organisms have endogenous defense mechanisms against reactive oxygen species, in which antioxidants play a major role. In the liver, glutathione is an important antioxidant. It removes hydrogen peroxide by converting reduced glutathione to its oxidized dimmer and has shown to protect against ischemia and reperfusion injury. Other important antioxidants are superoxide dismutase and vitamin E.

During the early phase of reperfusion, disturbances of the microcirculation occur which can ultimately lead to the ‘no reflow’ phenomenon. The basis of these disturbances is damage to the sinusoidal endothelial cells, caused by ischemia and reactive oxygen species released during reperfusion. This leads to leukocyte-endothelial cell interactions via induction of leukocyte adhesion molecules such as intercellular adhesion molecule-1. Also, procoagulant factors such as tissue factor and factor V and VII are released and activated, resulting in fibrinogen deposition and platelet adhesion. Furthermore, sinusoidal endothelial cell damage leads to impaired endothelium-dependant vasodilatation, while the potent vasoconstrictor endothelin-1 is produced during reperfusion. These factors result in endothelial edema, neutrophil and platelet plugging, microthromboses and vasoconstriction, ultimately leading to reduced microvascular circulation, hypoperfusion and decreased liver function.

2.2.3 Late phase of ischemia and reperfusion injury

The late phase of ischemia and reperfusion injury is characterized by the onset of an inflammatory reaction. Activation of Kupffer cells during the early phase results in the release of the proinflammatory cytokine tumor necrosis factor-α. Tumor necrosis factor-α has local damaging effects and plays a role in the induction of apoptosis. Also, tumor necrosis factor-α can cause expression of other proinflammatory cytokines, such as interleukin-1 and interleukin-6. This, together with the release of other mediators, such
as chemokines interleukin-8 and cytokine-induced neutrophil chemoattractant, leads to activation, attraction and transendothelial migration of neutrophils. Neutrophils release cytotoxic enzymes such as myeloperoxidase and elastase. Also, distant organ damage and inflammation can occur, as part of the systemic inflammatory response syndrome. This usually starts in the lungs and can lead to multiple organ failure.

### 2.2.4 Prevention of hepatic ischemia and reperfusion injury

Many animal studies have been performed, aiming at reduction of hepatic ischemia and reperfusion injury. Pharmacological interventions have been examined, focusing on different aspects of ischemia and reperfusion injury, such as scavenging reactive oxygen species, decreasing apoptosis or inhibiting the complement or coagulation cascade. In humans however, not many options have become available for treatment of the ischemia and reperfusion induced systemic inflammatory response syndrome. Prevention of inflammatory responses is another possible way to diminish I/R injury. Interleukin-10 is a potent anti-inflammatory cytokine, which inhibits the production of proinflammatory cytokines such as tumor necrosis factor-α interleukin-6, released by activated monocytes, macrophages and neutrophils. Interleukin-10 also induces downregulation of chemokines in rats and humans. Furthermore, interleukin -10 blocks nuclear factor-kappa B DNA binding and activation. The effect of interleukin-10 administration has been studied in various models of ischemia and reperfusion injury. In most of these studies, interleukin-10 appears to have an attenuating effect on organ damage caused by ischemia and reperfusion. Besides mediating the inflammatory response, tumor necrosis factor-α and interleukin-6 are also involved in priming hepatocyte proliferation when functional liver mass is lost. Therefore, interleukin-10 may on the one hand protect hepatic function by diminishing inflammation, but on the other hand may have negative effects by decreasing the proliferative response of hepatocytes through its action via interleukin-6 down-regulation. In chapter 8, the effect of interleukin-10 administration is examined in a rat model of 60 minutes hepatic ischemia and reperfusion. Outcome parameters were parenchymal damage, hepatic and pulmonary inflammation and the balance between hepatocellular apoptosis and proliferation.

Another mediator that enhances damage and inflammation after hepatic ischemia and reperfusion as well as after partial liver resection is lipopolysaccharide. It is released by Gram-negative bacteria, which migrate through the intestinal mucosa into the portal circulation and lymph nodes. This bacterial translocation is increased during and after hepatic ischemia-reperfusion and after partial liver resection. Lipopolysaccharide can be dephosphorylated and thereby inactivated by exogenous alkaline phosphatase. Alkaline phosphatase reduced inflammatory responses in terms of reduced cytokine response and neutrophil influx in sepsis and secondary peritonitis, two conditions in which lipopolysaccharide plays a prominent role. In chapter 9, the effect of exogenous administration of alkaline phosphatase is studied in a rat model of hepatic ischemia-reperfusion and resection. Outcome parameters were hepatic damage, pulmonary damage and inflammation.
PART 3

3.1 Hypothermic perfusion

Next to pharmacological interventions, other means to reduce or prevent hepatic ischemia and reperfusion injury have been explored. One of these techniques is in situ hypothermic perfusion of the liver. With this technique, the liver is cooled during ischemia via hepatic arterial or portal venous perfusion with a cold solution. Cooling of the liver results in decreased oxygen and energy demand owing to a lower rate of metabolism. Cellular metabolism is reduced 2-fold when temperature is lowered by 10°C. Therefore, cellular viability can significantly be prolonged during hypothermia. Hypothermic perfusion can only be applied during total hepatic vascular exclusion and not during Pringle's maneuver, because during total vascular exclusion hepatic venous drainage is blocked, preventing perfusion solution to flow via the caval vein into the systemic circulation. Hypothermic perfusion has shown to be effective in experimental as well as in clinical studies of liver resection.

From organ preservation studies it is known that parenchymal liver cells and sinusoidal endothelial cells respond differently to hypothermic conditions. Parenchymal cells and sinusoidal endothelial cells are both damaged after prolonged periods of warm ischemia (37°C), whereas cold ischemia (4°C) mostly affects sinusoidal endothelial cells and is associated with cell vacuolization and necrosis. Therefore, the temperature at which the liver is optimally protected during ischemia lies somewhere between 4°C and 37°C. In previous studies from our laboratory, using a partial hepatectomy model in the pig, it was found that hypothermic perfusion during total vascular exclusion at subnormothermic temperature (28°C), attenuated hepatocyte damage compared to perfusion at 37°C or no perfusion at all, and that sinusoidal endothelial cell function was spared. The question then arises whether hypothermic perfusion below 28°C further improves liver protection, bearing in mind that cold storage of liver grafts prior to transplantation is undertaken at 4°C.

In chapter 10, the effect of hypothermic perfusion at 37°C, 28°C and 20°C was examined in a pig model of 60 minutes total vascular exclusion. Pigs cannot withstand prolonged periods of elevated splanchnic pressure. Therefore, a shunt was used in all animals to bypass blood from the portal vein and inferior caval vein to the superior caval vein. Groups were compared for hepatic damage and inflammation as well as function of parenchymal cells and sinusoidal endothelial cells.

3.1.2 Organ preservation solutions

While Ringer-lactate is the most commonly used solution for hypothermic perfusion, organ preservation solutions also have been used. The advantage of organ preservation solutions lies in their ability to prevent tissue acidosis, cell swelling, free radical damage and energy depletion which occur during and after ischemia. University of Wisconsin solution is the gold standard organ preservation solution at this moment. Although effective,
the high viscosity and high potassium concentration of University of Wisconsin solution are considered a disadvantage, particularly in the setting of in situ hypothermic perfusion. Histidine-tryptophane-ketoglutarate, another organ preservation solution developed in Germany, has shown to be as effective as University of Wisconsin solution in preserving liver and pancreatic grafts. A relatively new organ preservation solution, called Celsior, combines the key components of both University of Wisconsin solution and Histidine-tryptophane-ketoglutarate solution. Celsior was originally designed for donor heart and lung preservation, but has also proven to be effective for preservation of kidney and liver grafts. In chapter 11, Celsior solution was compared to University of Wisconsin solution, Histidine-tryptophane-ketoglutarate solution and Ringer-lactate solution in a pig model of carotid artery preservation. Outcome parameters were vascular contraction and relaxation function. In chapter 12, hypothermic perfusion was performed at 28°C using Celsior solution and Ringer Lactate in a pig model of 60 min total vascular exclusion. Groups were compared for tissue acidosis, reactive oxygen species scavenging capacity, hepatic damage and coagulation.

Reference list


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